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Research report

Ventral medial prefrontal cortex and emotional perseveration: the memory for prior extinction training

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Abstract

Several years ago, we found that lesions of ventral medial prefrontal cortex (mPFCv) disrupted performance during the extinction component of a classical fear conditioning task without affecting acquisition performance. We called this emotional perseveration, hypothesizing that mPFCv may normally act to inhibit fear responses to a conditioned stimulus (CS) when the CS no longer signals danger. Subsequent studies have supported this hypothesis, showing that mPFCv is crucial for the memory of prior extinction training. The present study examined the effects of mPFCv lesions made after training. Such lesions resulted in reduced freezing to contextual stimuli and normal responding to the CS presented alone during a retention test. Rats were then subjected to extinction trials (CS without US) over multiple days. In contrast to pre-training lesions, post-training lesions had little effect on extinction rate. All rats were given additional training. Lesioned rats expressed greater fear reactions than controls, indicating that prior extinction was less effective in them. Lesioned rats also showed resistance to extinction during reextinction trials, confirming our earlier finding that lesions made before training weaken the effectiveness of extinction trials. These results suggest three conclusions. First, an intact mPFCv during acquisition may protect the animal from prolonged responding during extinction trials following brain insult. Second, changes in mPFCv may predispose subjects toward enhanced fear reactions that are difficult to extinguish when reexposed to fearful stimuli, due to a diminished capacity to benefit from the fear-reducing impact of prior extinction experience. Third, contextual cues processed by mPFCv may influence extinction performance.

Keywords: MPFCv; Fear conditioning; Extinction; Inhibition; Context; Reacquisition

1. Introduction

A prominent feature of prefrontal cortex (PFC) pathology is perseveration, the inability to inhibit behaviors that are no longer appropriate under present circumstances ([10,23,29,30,37,51,56]; see [31,32]). While such perseverative behaviors have typically been observed in reversal learning tasks (see [21,31]), several years ago we found evidence of perseveration in conditioned fear [40]. In a study examining the role of the ventral portion of medial PFC (mPFCv) in fear conditioning, lesioned rats exhibited emotional perseveration—an increased tendency to continue responding to a conditioned fear stimulus (a tone) in the absence of the unconditioned stimulus (US; foot shock) during extinction trials. We concluded that mPFCv plays an important role in regulating fear inhibition during the extinction process, when conditioned responses are weakened as a result of exposure to the conditioned stimulus (CS) alone.

Since that time, several studies have provided support for the idea that medial prefrontal cortex is involved in the extinction component of conditioned fear learning ([1,24,25,38,41,50]; but see [22]). Morrow et al. [41] found that mPFCv lesions disrupt extinction performance, whether lesions were made prior to or following acquisition training. The work of Quirk and colleagues [38,50] has been particularly informative in showing that mPFCv, the infralimbic cortex (IL) in particular, is important for the retention of extinction learning following a 24 h delay, but not during fear acquisition or the expression of extinction during massed extinction training. Their findings provide evidence for the prevailing idea that the extinction process involves formation of a new, inhibitory association that develops in competition with, but without erasure of, the excitatory association formed during acquisition [5,33,42,45,46,50].

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The current study had two objectives. The first was to examine the impact of post-acquisition lesions on the retention and extinction of fear responses. Previously, we found that pre-training lesions had no effect on the retention of acquisition but did affect extinction [40]. If mPFCv is only actively engaged during extinction retention [38,50], then rats with post-acquisition lesions should show resistance to extinction just as they did when lesions were made prior to fear conditioning [40]. The second goal was to examine the effectiveness of extinction by determining the extent to which reacquisition of fear responses is affected by prior extinction. Bouton and King [8] have proposed that memories of acquisition and extinction are both available following extinction, and that responding to an extinguished CS may be affected by both. Using the reacquisition procedure, it was possible to examine whether lesioned and control rats were differentially affected by extinction. We hypothesized that lesioned animals would express more fear than controls during reacquisition, which would indicate that prior extinction trials were less effective in guiding their behavior.

2. Materials and methods

2.1. Animals and general procedures

Male Sprague–Dawley rats, weighing 250–275 g upon arrival, were housed in pairs for 9 days in a colony room where they had unlimited access to rat chow and water and were exposed to a 12-h light:12-h dark cycle. Six days after arrival, animals received 1 day of context habituation and 2 days of acquisition training (described in the following). The next day they underwent surgery. Animals were assigned to one of two surgical groups based on training performance, such that the mean freezing scores to the context and CS were equivalent for the two groups. Following surgery and a 14-day recovery period, they received extinction trials to criterion (described in the following). Two to three minutes after the final extinction trial, they received an unsignaled, "reinstating" US, and the following day extinction trials commenced again to criterion. Reacquisition training began the next day, and was again followed by extinction trials to criterion.

2.2. Surgery

Animals were divided into two surgical groups: mPFCv lesions ($n = 17$), and controls ($n = 13$). The lesion group animals were anesthetized with Ketamine (100 mg/kg) and Xylazine (5 mg/kg) and placed in a stereotaxic frame. The skull was exposed and a hole over the mPFC was made using a dental drill. Coordinates (in mm relative to the interaural line) were AP = 12, ML = 0.6, and DV = 4.8 [47]. An epoxy-coated, stainless steel insect pin (500 μ exposed tip) was lowered into the brain and anodal constant current (1 mA) was passed for 10 s. All lesions were aimed bilaterally at the prelimbic/infralimbic (PL/IL) cortical region. The electrode was removed, the wound was sutured, and each animal was put in his own home cage and returned to the colony room to recover. Control animals were treated in the same way except that no electrode was used. All rats were housed individually for the remainder of the experiment, which recommenced 2 weeks after surgery.

2.3. Apparatus and behavioral procedures

2.3.1. Acquisition and extinction

The apparatus and procedures have been described elsewhere [39,48] and will be summarized here. All training and testing took place in a single conditioning box contained within a sound attenuating chamber. The CS was delivered through a speaker located below a continuously illuminated house light, both mounted on the front wall of the conditioning box. The US was delivered through a grid floor attached to a grid floor shocker. CS and US delivery were controlled by a personal computer. The sound attenuating chamber contained a peep hole in its door through which the experimenter observed the rat's activity. The experimenter was blind to the lesion status of each animal during all phases of training and testing.

Prior to training or testing on each day, rats were brought, in their home cages, to a holding room where they remained for 20–55 min (depending on running order, which was randomly assigned each day) before training/testing began. Experimentation began on Day 0 with a 20 min period of exposure to the conditioning box, during which the computer and all other equipment were turned on but no explicit CS or US was presented. Days 1 and 2 of the experiment were conditioning days, and consisted of two CS–US pairings on each day. The rat was placed in the chamber, and 90–210 s later the pre-CS (context measurement period) began. After 20 s, the CS (20 s, 10 kHz, 80 dB tone) was presented and coterminated with the US (0.5 s, 0.5 mA shock delivered through the grid floor). Trial 2 was the same. Thirty seconds after the offset of the second CS/US, the rat was removed from the conditioning chamber, placed in his home cage, and transferred to the outer room, where he remained until testing was completed on all rats. The chamber was cleaned with soap and water and thoroughly dried and aired out between each rat. The freezing response was used as the measure of conditioned emotional responding [3,7,19,36], and was assessed by observing the animal's behavior in the conditioning box. Stop watches were used to time the total amount of freezing, which was measured during the 20 s prior to the CS and during the 20 s CS to obtain measures of conditioned fear to the context in which conditioning took place and to the explicit CS. Only data from the first trial of each day were used as the measure of the effects of conditioning trials from the previous day. Twenty-four hours after conditioning, animals underwent surgery (see above), and were then allowed to recover in their home cages for 2 weeks.

Following recovery, animals were run on extinction trials, which were the same as conditioning trials except that the US was never presented. The first of these extinction trials was used as the post-surgical test of acquisition retention. Extinction trials continued, with two CS presentations a day, until animals reached the criterion for extinction, set at two consecutive days of 5 s or fewer spent freezing during the pre-CS and CS periods.

2.3.2. Reinstatement

Approximately 2.5 min after each rat had reached extinction criterion during the just prior extinction phase, he received an unsignaled US foot shock, which was of the same intensity as during original training but lasted for 1 s. The rat was then returned to his home cage. The following day the rat was returned to the conditioning chamber and extinction trials commenced to criterion in the same manner as during initial extinction trials.

2.3.3. Reacquisition and reextinction

The day after each rat reached extinction criterion, reacquisition training began. Reacquisition followed the same procedures as initial acquisition (two CS–US pairings a day for 2 days) for approximately half the rats (controls: $n = 6$, group *control-delay*; lesions: $n = 8$, group *mPFCv-delay*), and was again followed by a 2-week delay prior to extinction trials to mimic the initial post-surgical recovery period and thus control for the effect of this delay on extinction performance. The remaining rats (controls: $n = 7$, group *control-no-delay*; lesions: $n = 9$, group *mPFCv-no-delay*) received only 1 day of reacquisition training, and were started on reextinction trials the following day, to criterion. We eliminated the 2-week delay between acquisition and extinction trials for these animals to control for any effects the delay might have on rate of extinction; this was done in part to mimic the procedures of the original study [40], which had no delay between acquisition and extinction trials. These animals received only 1 day of reacquisition training to minimize the possibility of a ceiling effect and thus enhance the likelihood of seeing a difference between groups if rapid reacquisition were to occur.

2.4. Histology

Following completion of all behavioral testing, rats were given an overdose of chloral hydrate (4%, 1 cc/100 g) and were perfused with 100 ml of saline followed by 500 ml of 10% buffered formalin. Brains were removed from the skull and post-fixed in buffered formalin with 15% sucrose. Brains were then frozen and cut into 40-μm sections with a cryostat, with every fourth section before and after the lesion site and every section through the lesion site mounted on acid cleaned gelatin-coated slides. All mounted tissue was then stained with thionin (0.5%). Lesion placement was verified by microscopic examination, and all lesion boundaries were traced.

3. Results

3.1. Histology

All lesions included damage to PL and IL cortices. Damage to caudal medial orbital (MO) cortex was variable and did not produce any consistent behavioral changes on any measures, and thus was not used as a criterion for exclusion. One rat (group *mPFCv-no-delay*) received extensive damage to the overlying dorsal anterior cingulate cortex. Due to findings from a previous study [39] showing that such lesions enhance extinction effects by increasing fear levels, this animal was excluded from statistical analyses. See Fig. 1.

3.2. Behavior

3.2.1. Acquisition

The amount of freezing elicited by exposure to the context and to the CS over days 1 and 2 was used to measure fear acquisition prior to surgery (see Fig. 2). Animals were divided into lesion and control groups based on this performance, and the following analysis was conducted to ascertain that normal acquisition had taken place, and that the two groups did not differ prior to surgery. A $2 \times 2 \times 2$ ANOVA of surgical group (to-be-lesioned versus controls) by stimulus type (context versus CS) by day (1 and 2) produced significant main effects of stimulus type [$F(1, 27) = 24.687$; $P < 0.001$] and day [$F(1, 27) = 200.184$; $P < 0.001$] and an interaction of stimulus type by day [$F(1, 27) = 22.344$; $P < 0.001$]. No other effects were significant. As is typical of the acquisition of this task [40,48], this shows that animals froze more on day 2 than day 1 and froze more to the CS than to the context, indicating that acquisition had taken place. There were no differences in freezing between the two groups prior to surgery.

3.2.2. Retention test

The amount of freezing during the first extinction trial, 2 weeks after training and surgery, was used to assess the effects of the lesion on previously acquired conditioned responses (see Fig. 2). A 2×2 ANOVA of surgical group by stimulus type at test showed a main effect of stimulus type [$F(1, 27) = 23.77$; $P < 0.001$] and an interaction of surgical group by stimulus type [$F(1, 27) = 7.413$; $P < 0.05$]. A post hoc *t*-test showed that lesioned animals froze significantly less to the context than did controls [$t(27) = 2.53$; $P < 0.05$]. In sum, following surgery, lesioned animals responded at the same level as controls to the CS, but responded less than controls to the context. Thus, mPFCv lesions did not affect previously established fear responses to the CS but disrupted retention of contextual conditioning.

3.2.3. Extinction

The measure used to examine extinction was number of days to reach extinction criterion, set at two consecutive days of 5 s or fewer spent freezing during the pre-CS period

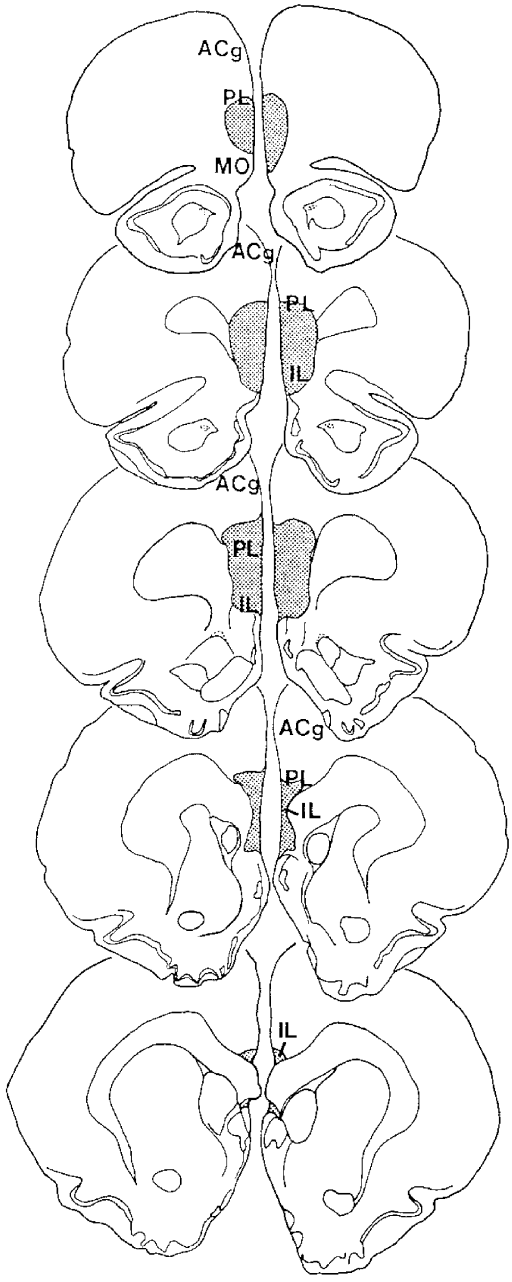

Fig. 1. Representational sections, from rostral to caudal, of a ventral medial prefrontal cortex (mPFCv) lesion, displaying the full rostro-caudal extent of the lesions. mPFCv as defined here includes the PL and IL cortical areas lying along the medial wall of the frontal lobe rostral to the genu of the corpus callosum, largely sparing adjacent cortical regions. Lesioned regions are depicted here with stippling. The areas depicted include gliosis and the lesion cavity. ACg = anterior cingulate cortex; PL = prelimbic cortex; MO = medial orbital cortex; IL = infralimbic cortex. Cortical delineations from Paxinos and Watson [47].

(context test) and during the CS period. A 2 × 2 ANOVA of surgical group by stimulus type produced a main effect of stimulus type [$F(1, 27) = 52.64$; $P < 0.001$], showing that all animals took longer to extinguish responding to the CS than to the context, as is typical on this task. No other factors reached statistical significance. Due to the finding of prolonged responding by mPFCv-lesioned animals to the CS in our previous study [40], we examined days to extinction for the CS alone; it was short of statistical significance [$t(27) = 1.779$; $P = 0.086$]. Thus, lesioned animals did not differ significantly from controls in their rate of extinction to either the context or CS, though there was a trend towards prolonged responding to the CS by lesioned animals (see Fig. 3).

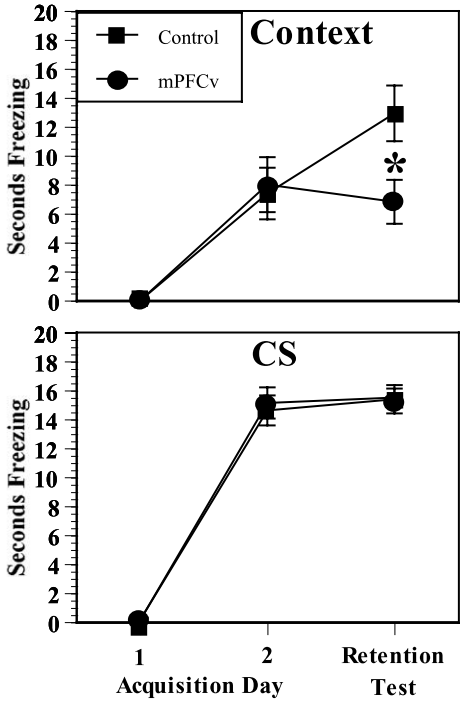

Fig. 2. Acquisition of fear. Mean number of seconds spent freezing during the 20 s prior to the onset of the CS (context test) and during the 20 s CS are shown. Animals received two tone (CS)–shock (US) pairings on days 1 and 2, underwent surgery the next day, and began extinction trials following a 2-week recovery period. Freezing responses during the first trial of days 1 and 2 reflect fear conditioning prior to surgery. Freezing responses during the first extinction trial, the post-surgical test of acquisition retention, reflect the effects of mPFCv lesions on fear conditioning. Asterisk (∗) indicates that lesioned animals froze significantly less than did controls during the post-surgical test of contextual conditioning ($P < 0.05$).

3.2.4. Reinstatement

This procedure was ineffective in reinstating a CR to the CS for all animals. Freezing levels to the context and CS measured 24 h after animals received an unsignaled US were minimal and did not differ for control and lesioned animals; thus, the majority of animals in both groups reached extinction criterion within the procedurally obligatory 2 days. The mean increase in freezing following exposure to the US alone was: Controls – context = 4.77 s, CS = 4.08 s; mPFCv – context = 5.69 s, CS = 4.44 s.

3.2.5. Reacquisition

All animals received two tone–shock pairings on the first day of reacquisition. On the second day, roughly half the animals (groups *control-delay* and *mPFCv-delay*) again re-

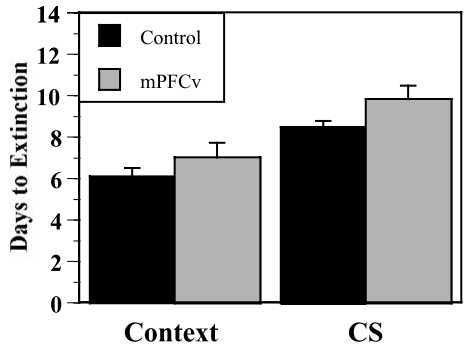

Fig. 3. Extinction of fear. Mean number of days to reach criterion is shown. Extinction criterion was defined as 5 s or fewer of freezing during the context test period and during the CS on two consecutive days. Lesioned animals only approached significance in extinction of responding to the CS ($P = 0.086$).

ceived two tone–shock pairs, while the other half (groups *control-no-delay* and *mPFCv-no-delay*) received two presentations of the tone alone (i.e. they were started on extinction trials). As in the analysis from initial acquisition, we examined freezing behavior elicited by exposure to the context and to the CS on the first trial of day 1 and day 2 to measure the effects of training from the prior day (see Fig. 4). All animals (both the delay and no-delay groups) were included in this analysis since they received identical treatments until the last 500 ms of trial 1 on day 2. A $2 \times 2 \times 2$ ANOVA of surgical group by stimulus type by day produced significant main effects of surgical group [$F(1, 27) = 11.237$; $P < 0.01$] and day [$F(1, 27) = 108.811$; $P < 0.001$], and a significant interaction of surgical group by day [$F(1, 27) = 14.983$; $P < 0.01$]. Lesioned animals froze substantially more to both the context and CS than did controls on day 2.

3.2.6. Retention test

Two groups of animals (*control-delay* and *mPFCv-delay*) underwent a 2-week delay between reacquisition and reextinction trials to mimic the 2-week post-surgical recovery period following acquisition in phase one. We measured freezing levels during the first trial following the delay to assess the effect of the delay on reacquisition of conditioned responding for these two groups. A 2×2 ANOVA of surgical group by stimulus type at test produced only a significant main effect of surgical group [$F(1, 12) = 23.42$; $P < 0.001$], indicating that lesioned animals continued to freeze more than controls following the delay, even to the context. In sum, the reacquisition results show that lesioned animals responded more than controls to both the context and CS during reacquisition. Further, not only were lesioned animals capable of freezing to the context, but they continued to freeze more than controls even after the delay.

3.2.7. Reextinction

Comparing groups *control-no-delay* and *mPFCv-no-delay* on days to reextinction, we found that lesioned animals took

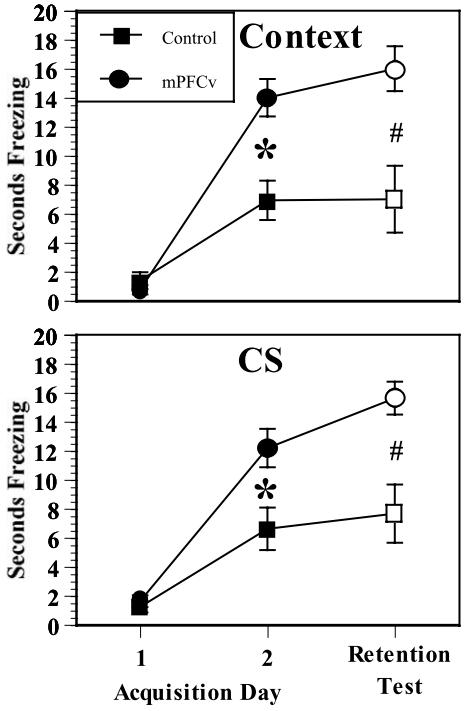

Fig. 4. Reacquisition of fear. Mean number of seconds spent freezing during the 20 s prior to the onset of the CS (context) and during the 20 s CS are shown. All animals received two tone (CS)–shock (US) pairings on the day following completion of extinction training of the prior experimental condition. Groups *control-delay* and *mPFCv-delay* received a second day of tone (CS)–shock (US) pairings and began extinction trials following a 2-week delay, replicating the procedures from initial acquisition. Day 2 reflects the effects of conditioning from day 1 for all animals. Data from the first extinction trial (retention test) reflect the effects of a 2-week delay on fear conditioning, and thus are presented only for groups *control-delay* and *mPFCv-delay* (open symbols). Asterisk (∗) indicates that lesioned animals responded significantly more than did controls during reacquisition of both the context and CS (day 2, all animals; $P < 0.01$), (#) even following a 2-week delay (retention test; groups *control-delay* and *mPFCv-delay*; $P < 0.05$).

longer than controls to extinguish the freezing response (see Fig. 5A). A 2×2 ANOVA of surgical group by stimulus type produced significant main effects of surgical group [$F(1, 13) = 9.816$; $P < 0.01$] and stimulus type [$F(1, 13) = 5.220$; $P < 0.05$], and a significant interaction of stimulus type by surgical group [$F(1, 13) = 6.112$; $P < 0.05$]. Planned comparisons done separately for the context and CS showed that the effect of surgical group was significant for the context [$t(13) = 2.196$; $P < 0.05$] and also for the CS [$t(13) = 3.463$; $P < 0.01$]. Thus, lesioned animals now showed resistance to extinction to both the context and CS, but relatively more to the CS than to the context.

Comparing groups *control-delay* and *mPFCv-delay* (the animals which underwent identical treatments during acquisition and reacquisition), lesioned animals took longer to extinguish the freezing response than did controls, and in the same pattern as the no-delay groups (see Fig. 5B). A 2×2 ANOVA of surgical group by stimulus type produced a significant main effect of surgical group [$F(1, 12) = 35.335$;

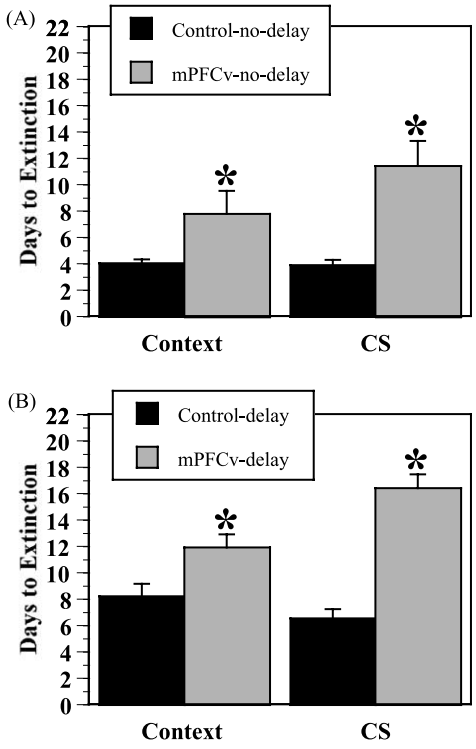

Fig. 5. Reextinction of fear. Mean number of days to reach extinction criterion is shown for animals receiving: (A) 1 day of tone (CS)–shock (US) pairings immediately followed by reextinction trials (groups *control-no-delay* and *mPFCv-no-delay*); (B) two days of tone (CS)–shock (US) pairings followed by a 2-week delay before beginning extinction trials (groups *control-delay* and *mPFCv-delay*). Asterisk (∗) indicates that lesioned animals took significantly longer to reextinguish than did controls to both the context ($P < 0.05$) and CS ($P < 0.01$).

$P < 0.001$] and an interaction of stimulus type by surgical group [$F(1, 12) = 11.561$; $P < 0.01$]. Planned comparisons done separately for the context and CS showed that the effect of surgical group was significant for the context [$t(12) = 2.54$; $P < 0.05$] and for the CS [$t(12) = 6.776$; $P < 0.001$]. Again, lesioned animals now showed resistance to extinction to both the context and CS, while the interaction shows that lesioned animals took significantly longer to extinguish responding to the CS than to the context relative to controls.

3.2.8. Acquisition versus reacquisition/extinction versus reextinction

Since lesioned animals froze more than controls during reacquisition, it is not surprising that they showed resistance to extinction relative to controls during reextinction. Thus, in order to establish a more comparable acquisition baseline from which to examine reextinction performance, we compared group *mPFCv-delay*'s reacquisition performance with their own initial acquisition performance, since they underwent identical behavioral procedures during acquisition and reacquisition. Conducting separate *t*-tests for the context and for the CS on day 2, the results were significant

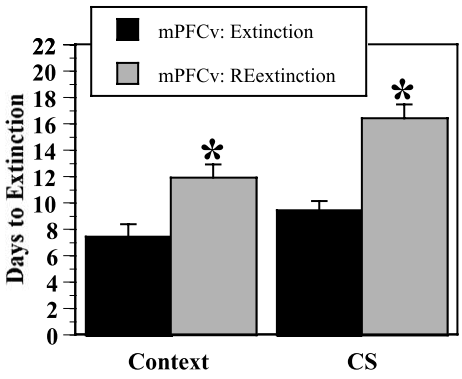

Fig. 6. Days to extinction and reextinction are compared for lesioned animals receiving the same procedures during and after acquisition and reacquisition (*mPFCv-delay*). Asterisk (∗) indicates that lesioned animals (group *mPFCv-delay*) took significantly longer to extinguish responding during reextinction than during extinction ($P < 0.01$).

for the context [$t(7) = 3.259$; $P < 0.05$], but not for the CS. This indicates that lesioned animals froze more to the context during reacquisition than acquisition, but responded equivalently during both sessions to the CS.

We can now be sure that any resistance to extinction seen during reextinction relative to initial extinction cannot be attributed to greater levels of freezing to the CS during reacquisition relative to initial acquisition. For lesioned animals in group *mPFCv-delay*, a 2 × 2 ANOVA of session (extinction versus reextinction) by stimulus type produced significant main effects of session [$F(1, 7) = 15.424$; $P < 0.01$] and stimulus type [$F = 20.012$; $P < 0.01$], and a significant interaction ($F = 7.875$; $P < 0.05$). This indicates that lesioned animals took longer to extinguish the conditioned response during reextinction than they did during extinction, and that this effect of prolonged responding during reextinction was relatively greater to the CS than to the context (see Fig. 6).

3.2.9. Fear conditioning summary

In sum, when lesioned following acquisition training, mPFCv-lesioned animals responded significantly less to the context than did controls, but responded normally to the CS. Lesioned animals extinguished responding at about the same rate as controls to the context, and had a non-significant trend towards prolonged responding to the CS. Upon reacquisition, lesioned animals responded significantly more than control animals to both the context and CS, and continued to do so after a 2-week delay. Lesioned animals responded more to the context during reacquisition than they did during initial acquisition, but they responded at about the same level to the CS during both sessions. Upon reextinction, lesioned animals showed resistance to extinction to both the context and CS relative to controls, particularly to the CS. Lesioned animals also showed resistance to extinction during reextinction relative to their own performance during the initial extinction session.

4. Discussion

4.1. Acquisition

In the present study we made lesions of mPFCv following acquisition training but prior to extinction. On the test of retention 2 weeks after surgery, lesioned animals responded like controls to the CS, but responded significantly less to the context. mPFCv receives a prominent projection from the hippocampal formation [12,27,28], lesions of which also greatly reduce responding to the context without affecting CS conditioning [48]. However, when we made lesions prior to training [40] we found no lesion effects on responding to contextual cues, which one would expect to see if mPFCv were involved in the acquisition or expression of contextual conditioning. Administering the lesion between acquisition and testing may have functionally changed the context to the extent that some of the relevant cues associated with contextual excitation were not available at the time of testing.

4.2. Extinction

We found a slight, non-significant trend of resistance to extinction of the CR to the CS. This is in contrast to a robust and prolonged resistance to extinction effect found previously when lesions were made prior to acquisition. Due to our a priori directional hypothesis of prolonged responding to the CS by lesioned animals, a one tailed *t*-test would have been an appropriate analysis, and would have produced a $P < 0.05$; however, this would not change the marginal nature of the effect. These results suggest that mPFCv may influence extinction performance through processes occurring during acquisition rather than only during extinction. Although recent studies suggest that mPFC is not influential or active during acquisition [38,50], this should be explored further. Another possibility is that an intact mPFCv during acquisition may protect the animal from prolonged fear. This region may normally be involved in providing information to other brain regions, which allows them to subsequently update response output. Even in the original study [40], mPFCv animals eventually extinguish the CR. This alternative possibility is consistent with the literature suggesting that if a brain region is intact during initial exposure to a situation, then the animal may be protected from the deleterious effects of subsequent brain trauma [53].

4.3. Reacquisition

The reacquisition procedure allowed us to examine the effect of prior extinction training on further conditioning. Bouton and King [8] have proposed that memories of acquisition and extinction are both available following extinction, and that responding to an extinguished CS may be determined by whether a memory of acquisition or extinction is active. During reacquisition, responding should recover more rapidly when conditions are more reminiscent of conditioning than of extinction [4]. With the hypothesis that lesioned animals are deficient in the use of an inhibitory association developed during extinction, we used reacquisition to examine if lesioned animals would demonstrate an increased rate of reacquisition relative to controls after prior extensive extinction training. Lesioned animals froze more than controls to both the context and the CS during reacquisition, suggesting that mPFCv may normally be involved in inhibitory functions. It may also be that measuring the degree of fear elicited during post-extinction reacquisition is a more sensitive measure of extinction than the usual approach of measuring fear responses elicited by the CS over extinction trials. In support of our hypothesis, a number of studies have suggested that mPFC has generally inhibitory functions [10,15,17,29,31,37], which have been demonstrated in lesion studies as a decreased ability to inhibit responding when that response is no longer appropriate to the present situation ([10,16,37]; and see [11]).

The reacquisition findings are similar to the findings of Quirk et al. [50], despite major differences in behavioral procedures. They found that animals with mPFCv lesions performed like controls during massed CS acquisition and extinction trials presented on day 1. However, during the test of recovery the following day, controls showed little freezing to the tone, while lesioned animals showed substantial recovery of the freezing response, performing similarly to a control group which had never received extinction training. These results suggest that mPFCv is necessary for the recall of extinction learning following a 24-h delay, but not for the expression of initial extinction. Similarly, in a recording study [38], they found that cells in mPFCv were active only during extinction recall, not during the acquisition or extinction training trials on the previous day. These findings are compatible with our procedure of extinction trials spaced over several days, in that extinction recall would be a component of extinction performance during extinction trials on successive days. These results lend strong support to the hypothesis that mPFCv-lesioned animals are deficient in the use of an inhibitory association developed during extinction.

4.4. Reextinction

After reacquisition, we carried out additional extinction trials. The findings from this part of the study confirm our earlier findings [40] that mPFCv lesions made before training prolong the extinction of conditioned responding. Further, the resistance to reextinction effect displayed by group *mPFCv-delay* (those lesioned animals which received a 2-week delay between reacquisition and reextinction trials to mimic the delay following surgery) indicates that it was not the 2-week delay between acquisition and extinction that was responsible for any protection from prolonged responding during initial extinction. Both lesion groups *(mPFCv-delay* and *mPFCv-no-delay*) took longer to extinguish than did controls (*groups control-delay* and *control-no-delay*).

If we assume that levels of freezing during acquisition are related to the strength of excitatory conditioning, and thus to rate of extinction, then we would indeed have expected lesioned animals to show prolonged responding during reextinction relative to controls: they froze more during reacquisition. Thus, we also compared group *mPFCv-delay*'s reextinction performance with their own initial extinction performance since they commenced extinction training in both cases at equivalent levels of conditioned freezing to the CS. These animals reached equivalent peak mean freezing levels on day 3 to the CS during both acquisition (16.625 s) and reacquisition (15.625 s). Yet they took far longer to give up responding during reextinction than during initial extinction. Interestingly, while they responded more to the context during reacquisition than acquisition, the prolonged responding during reextinction relative to extinction was far more pronounced to the CS than to the context. This may suggest a role for context in influencing responding to the CS during extinction.

4.5. General discussion

How might mPFCv be involved in regulating extinction? A prevailing idea about the associative functions underlying extinction is that an inhibitory association develops in competition with, but without erasure of, the excitatory association developed during acquisition [5,33,45]. A recent study using metabolic mapping to examine activity in the mouse brain after extinction of a conditioned fear response [1] supports this theory, and implicates IL cortex as the crucial portion of mPFCv involved in extinction; the work of others [38,50] also points to IL as the vital region. Our reacquisition and reextinction results suggest that mPFCv is normally involved in utilizing this inhibitory association that develops during extinction. It has been shown that the inhibitory association is less stable than the excitatory association, and is dependent on the presence of the extinction context for its expression (for review see [6,42]). During reacquisition, conditioning is more likely to be disrupted if the contextual cues are associated with extinction [9]. A number of researchers have suggested that mPFC may be involved in providing an internal emotional context [20,31,37,43,49]. mPFCv may influence contextual conditioning and extinction by helping to integrate information about the internal environment with the external environment, providing an emotional context via its amygdalar and visceral connections [26,54,55]and its inputs from the hippocampus. With a defect in this circuit such that the internal context is not being integrated with external events, the organism may have difficulty recognizing the context as that associated with inhibition (or safety); it will tend towards the cautionary behavior of continuing to freeze during subsequent extinction trials. This does not claim that lesioned animals are incapable of conditioning to the context, but only that they may be deficient in using it as a guide for utilizing an inhibitory association or in disambiguating the meaning of the CS. These speculations are in line with other theories of prefrontal function which suggest that PFC is involved in associating affective visceral cues with external events to allow for appropriate response output [2,13,43,44].

These experiments demonstrate for the first time that mPFCv-lesioned animals are deficient in the acquisition of conditioned responding specifically to contextual cues. They support earlier findings that mPFCv is involved in the inhibition of conditioned responding to an extinguished CS under certain conditions, while an intact mPFCv during acquisition may protect the animal from prolonged fear. Though speculative at this point, the results further indicate that mPFCv may utilize appropriate contextual cues to guide performance following extinction training. It is well established that the amygdala is crucial for the formation of the initial excitatory association between a CS and US (see [35] for review), and it has also been tentatively implicated in extinction. Davis and co-workers [18] found that blockade of NMDA receptors in the amygdala interferes with extinction of conditioned fear. mPFCv has significant connections with the amygdala, and with autonomic centers to which the amygdala also projects (see [34,52]). Perhaps mPFCv integrates visceral contextual cues with external contextual information from the hippocampus. This contextual information may be used most heavily during and after extinction to guide appropriate responding to the CS when its meaning may be most ambiguous. Further experiments examining more directly the role of mPFCv in contextual conditioning and in the excitatory and inhibitory stages of learning should provide greater insight into the neural basis of fear learning as a whole.

An important implication of these findings is that changes in mPFCv might predispose one to develop fear responses that are difficult to extinguish or otherwise treat. This could play a role in various psychiatric disorders involving fear and anxiety, especially given that imaging studies often show abnormal activity in this region in psychiatric patients. Further, recent studies in normal humans have found that functional activity in the mPFC and amygdala are inversely related, suggesting that mPFC may regulate fear responses mediated by the amygdala [14]. Subtle changes in the chemistry or connectivity of this region, due to stress or other factors, might therefore predispose people to retain fear experiences in ways that are difficult to eliminate.

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